DATA EVALUATION RECORD

1. **CHEMICAL:** Bromoxynil, Octanoate
Shaughnessey Number 035302

2. **TEST MATERIAL:** Bromoxynil Octanoate Technical; 2,6-dibromo-4-cyanophenyl octanoate; M & B Lot No. CN-51033 (20-DLM-152-1); Analytical Log No. 14542; 97.2% active ingredient; a brown solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Skeletonema costatum.

4. **CITATION:** Giddings, J.M. 1990. Bromoxynil Octanoate - Toxicity to the Marine Diatom Skeletonema costatum. Prepared by Springborn Laboratories, Inc., Wareham, Massachusetts. SLI Report #90-8-3440. SLI Study #10566-1089-6142-450. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, North Carolina. MRID Number 416060-02.

5. **REVIEWED BY:**

Kimberly Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Kimberly Rhodes
Date: February 11, 1991
Charles Lum 2/15/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 2/12/1991

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 2/19/91

7. **CONCLUSIONS:** Due to the problem with the test material solubility observed in other studies using the same chemical, this study cannot be properly validated until the measured concentrations (addendum to this study report) are submitted. With a 5-day EC50 value of 0.14 mg a.i./L nominal concentration and a 5-day NOEC value of 0.033 mg a.i./L nominal concentration, Bromoxynil is expected to exert a detrimental effect on the marine diatom (Skeletonema costatum) when applied at application rates up to 0.375 lbs a.i./A.

Upgraded to core
7 hrs

8. **RECOMMENDATIONS:** Tier III testing should be conducted since Bromoxynil is expected to exert a detrimental effect on the marine diatom (Skeletonema costatum) at the maximum application rate.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Organism:** The marine diatom (Skeletonema costatum) used in this toxicity test was originally obtained from Bigelow Marine Laboratory located in West Boothbay, Maine. The stock culture was maintained under test conditions at the testing facility.

Stock cultures were transferred to fresh medium approximately once or twice a week. The inoculum used to initiate the toxicity test with Bromoxynil was taken from a stock culture that had been transferred to fresh medium five days before testing.

The culture medium used was Marine Algal Medium prepared in a filtered natural seawater and adjusted to pH 8.0 $\pm$ 0.1 with 0.1N hydrochloric acid. Stock cultures were grown in 125-mL glass flasks containing 50 mL of medium. The flasks were covered with stainless steel caps which permitted gas exchange.

B. **Test System:** The phytotoxicity test was conducted in an environmental chamber where the temperature was maintained at 20-22°C. The test vessels were sterile 125-mL flasks fitted with stainless steel caps which permitted gas exchange. The flasks were impartially placed on an orbital shaker set at 60 rpm. Lighting was provided continuously at an intensity of 4,000-5,000 lux at the solution surface.

The marine algal medium used to prepare the exposure solutions was formulated in the same manner as the culture medium (excluding Na_4EDTA).

C. **Dosage:** Five-day growth and reproduction test. The nominal test concentrations of Bromoxynil based on active ingredient were 0.033, 0.065, 0.13, 0.25, and 0.50 mg/L.

D. **Design:** Based on the results of preliminary testing, a control, solvent control, and five nominal Bromoxynil concentrations (see Section 11.C) were selected for testing. The solvent control contained 0.1 mL/L of acetone which was equivalent to the concentration of solvent present in all Bromoxynil test solutions. Each concentration and control were replicated three times.

After the test solutions were added to the test flasks, an inoculum of Skeletonema costatum cells calculated to provide 1.0×10^4 cells/mL was aseptically introduced into each flask. The inoculum volume was 1,000 µL per flask. At each 24-hour interval, cell counts were conducted on each replicate vessel using a hemacytometer and a compound microscope. One sample was taken from each flask for counting. One or more hemacytometer fields, each 0.1 x 0.1 cm in surface area and 0.01 cm deep and containing 0.0001 mL of culture, were examined for each sample until at least 400 cells or four fields were counted; when cell densities were less than 100×10^4 cells/mL, fewer than 400 cells were present in four fields but no more than four fields were counted.

The pH of the test solutions was measured at test initiation and termination. Measurements at test initiation were conducted on the test solutions remaining in the 500-mL volumetric flasks after the test flasks had been filled. At test termination, the remaining test solution in each replicate of each test concentration were composited and a portion of the composite solution was transferred to a 100-mL beaker for pH and conductivity measurement. Temperature was measured continuously with a minimum/maximum thermometer in a flask of water placed next to the test vessels. The shaking rate of the orbit shakers was recorded daily. The light intensity of the test area was measured with a light meter at test initiation and each 24-hour interval of the exposure period.

At test initiation, samples for analysis were removed from the 500-mL volumetric flasks of the test solutions and the controls and frozen for analysis. In addition, six quality control (QC) samples were prepared. The results of the analysis of these QC samples were used to judge the precision and quality control maintained during the analytical process. All solutions were analyzed for Bromoxynil by high pressure liquid chromatography.

E. **Statistics:** EC10, EC50, and EC90 values and their 95% confidence limits were determined after 24, 48, 72, 96, and 120 hours of exposure by linear regression of response (percent reduction of cell density as compared with controls) vs. nominal concentration over the range of test concentrations where a clear exposure-response relationship was observed. Four linear regressions were estimated based on (a) untransformed data, (b) untransformed response vs. logarithm-transformed concentration, (c) probit-transformed response vs. untransformed concentration, and (d) probit-transformed response vs. logarithm-transformed concentration. The regression that best fitted the data was selected based on the highest coefficient of determination (r^2). This regression equation was then applied to estimate the EC values and their 95% confidence limits, using the method of inverse prediction.

A t-test was used to compare the controls with solvent controls. Comparison of controls with solvent controls indicated no significant difference (P = 0.01) in cell density. The data from the two sets of controls were therefore pooled for analysis. Before conducting the analysis, the data were checked for normality using the Chi-Square test and for homogeneity of variance using Hartley's Test. The no-observed-effect concentration (NOEC) was determined using one-way analysis of variance and Bonferroni's Test since treatment groups had unequal numbers of replicates (i.e., control data were pooled).

12. **REPORTED RESULTS:** The analytical method validation for Bromoxynil in marine algal medium was unsuccessful due to interferences caused by the seawater matrix. The samples are in frozen storage and will be analyzed when the method is validated. Results will be reported in an amendment to this report. Nominal concentrations were used for EC and NOEC calculations.

Cell densities determined at each observation time are presented in Table 2 (attached). Control cell densities averaged 145 x 10^4 cells/mL at 120 hours. Solvent control densities averaged 119 x 10^4 cells/mL at 120 hours. Cell densities increased over time in all replicates at concentrations $\leq$ 0.25 mg a.i./L and generally followed the concentration gradient established. No growth occurred in cultures exposed to 0.50 mg a.i./L. Cells in cultures exposed to 0.065 mg a.i./L were paler than normal. Cells in

cultures exposed to 0.13 and 0.25 mg a.i./L were more paler and cell fragments were also observed.

The 120-hour EC10 value, based on nominal concentrations, was determined to be 0.034 mg a.i./L with a 95% confidence interval of 0.016-0.067 mg a.i./L. The 120-hour EC50 value, based on nominal concentrations, was determined to be 0.13 mg a.i./L with a 95% confidence interval of 0.063-0.25 mg a.i./L. The 120-hour EC90 value, based on nominal concentrations, was determined to be 0.47 mg a.i./L with a 95% confidence interval of 0.24-1.0 mg a.i./L. The 120-hour NOEC was determined by Bonferroni's test to be 0.033 mg a.i./L nominal concentration.

At test initiation, pH was 8.2 in all test solutions. At test termination, pH had increased to 8.7 in all cultures where algal growth had occurred. Temperatures ranged from 20 to 22°C during the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the author.

The study was conducted following the intent of the Good Laboratory Practice Regulations and the study conduct, raw data and final report were reviewed by Springborn Laboratories, Inc. Quality Assurance Unit. A Quality Assurance Statement was included and signed by the Quality Assurance Manager.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

o The SEP states that a photoperiod of 16-hours of light and 8-hours of darkness with a light intensity of 4,000 lux should be used when testing Skeletonema costatum. During this test, a continuous photoperiod with a light intensity of 4,000 to 5,000 lux was maintained.

o The maximum application rate of the test substance was not provided in the report.

B. **Statistical Analysis:** The reviewer used the EPA's Toxanal computer program to calculate the 5-day EC50 value using cell density percent inhibition as the growth endpoint. These calculations are attached. Percent inhibition (I) of growth compared to the

solvent control was calculated for cell density according to the following formula:

$$\% I = \frac{C - T}{C} \times 100$$

where: C = mean growth in the control,
T = mean growth in test concentration.

The 5-day EC50 value, based on nominal concentrations, was determined to be 0.14 mg a.i./L with a 95 percent confidence interval of 0.13-0.16 mg a.i./L.

Analysis of variance with multiple comparison tests was performed to compare cell density at each treatment level to those of the solvent control for day 5 (attached). Cell density was significantly reduced in the four highest nominal concentrations (0.065, 0.13, 0.25, and 0.50 mg a.i./L) when compared to the solvent control. The 5-day NOEC value was determined to be the same as that reported by the author (0.033 mg a.i./L).

C. **Discussion/Results:** Due to the problem with the test material solubility observed in other studies using the same chemical, this study cannot be properly validated until the measured concentrations (addendum to this study report) are submitted. The 5-day EC50 value of Bromoxynil for Skeletonema costatum was determined to be 0.14 mg a.i./L nominal concentration. The 5-day NOEC was determined to be 0.033 mg/L nominal concentration.

The maximum application rate was not provided in this report, however, an accompanying algal report performed by Springborn Laboratories, Inc. (MRID #416060-04) reported that direct application of 0.375 lbs a.i./acre (A) to a one acre, 0.5 feet deep pond would result in an estimated Bromoxynil environmental concentration (EEC) of 0.275 mg a.i./L. This EEC value (0.275 mg a.i./L) is two times greater than the estimated 5-day EC50 value of 0.14 mg/L nominal concentration. Therefore, based on nominal concentrations, Bromoxynil is expected to exert a detrimental effect on the marine diatom (Skeletonema costatum) following normal application methods at rates up to 0.375 lbs a.i./A. Based on these results, a Tier III toxicity test is required.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Supplemental~~. Upgraded to core (see D170117)

(2) **Rationale:** Measured concentrations were not submitted as an addendum as reported.

(3) **Repairability:** Pending the evalutation of the chemical measurement of test solutions.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 01-25-91.

Page _____ is not included in this copy.

Pages 8 through 9 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

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____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

5-day EC50

KIMBERLY RHODES BROMOXYNIL SKELETONEMA COSTATUM 01-25-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.5	100	100	100	0
.25	100	53	53	0
.13	100	49	49	0
.065	100	24	24	0
.033	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1530786

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	8.83111E-03	.1440985	(.1324189 .1571321)

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.6998613	12.64361	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.748198
95 PERCENT CONFIDENCE LIMITS = .4491184 AND 5.047278

LC50 = .1512891
95 PERCENT CONFIDENCE LIMITS = 5.067595E-02 AND .5644848

LC10 = 5.220194E-02
95 PERCENT CONFIDENCE LIMITS = 2.253579E-04 AND .1057303

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5-day NOEC

ıalysis of Variance File: BROMSKE Date: 01-25-1991

ILTER: None

's, means and standard deviations based on dependent variable: CELLDEN

* Indicates statistics are collapsed over this factor

Factors: C	Nominal Concentration (mg a.i./L)	N	Mean	S.D.
*		21	84.4762	47.2119
1	Solvent control	3	119.0000	8.1854
2	Control	3	145.3333	7.7675
NOEC-3	0.033	3	119.0000	12.2882
ǂ 4	0.065	3	90.3333	4.0415
ǂ 5	0.13	3	61.3333	5.5076
ǂ 6	0.25	3	56.3333	9.2916
ǂ 7	0.50	3	0.0000	0.0000

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ımax for testing homogeneity of between subjects variances: Not defined

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

ıalysis of Variance Dependent variable: CELLDEN

ource	df	SS (H)	MSS	F	P
etween Subjects	20	44579.2380			
C (CONC)	6	43756.5700	7292.7617	124.107	0.0000
Subj w Groups	14	822.6680	58.7620		

ǂ Significantly different from the solvent control.

5-day NOEC

ıalysis of Variance File: BROMSKE Date: 01-25-199

ILTER: None

ɔst-hoc tests for factor C (CONC)

.evel	Mean	Level	Mean
1	119.000	6	56.333
2	145.333	7	0.000
3	119.000		
4	90.333		
5	61.333		

	Comparison	Tukey-A*	Newman -Keuls*	Bon- ferroni	Dunnett
*	1 < 2	0.0500	0.0100	0.0190	0.0100
NOEC-	1 = 3				
*	1 > 4	0.0100	0.0100	0.0095	0.0100
*	1 > 5	0.0100	0.0100	0.0000	0.0100
*	1 > 6	0.0100	0.0100	0.0000	0.0100
*	1 > 7	0.0100	0.0100	0.0000	0.0100
	2 > 3	0.0500	0.0100	0.0190	N.A.
	2 > 4	0.0100	0.0100	0.0000	N.A.
	2 > 5	0.0100	0.0100	0.0000	N.A.
	2 > 6	0.0100	0.0100	0.0000	N.A.
	2 > 7	0.0100	0.0100	0.0000	N.A.
	3 > 4	0.0100	0.0100	0.0095	N.A.
	3 > 5	0.0100	0.0100	0.0000	N.A.
	3 > 6	0.0100	0.0100	0.0000	N.A.
	3 > 7	0.0100	0.0100	0.0000	N.A.
	4 > 5	0.0100	0.0100	0.0086	N.A.
	4 > 6	0.0100	0.0100	0.0021	N.A.
	4 > 7	0.0100	0.0100	0.0000	N.A.
	5 > 6				N.A.
	5 > 7	0.0100	0.0100	0.0000	N.A.
	6 > 7	0.0100	0.0100	0.0000	N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000).
A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

* Significantly different from the solvent control.

Shaughnessy No. 035301 Chemical Name Bromoxynil Chemical Class ____ Page ____ of ____

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Va
1-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(X)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
1-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(X)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(X)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	
Acc.		Comments:		
-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(X)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(X)= Sol. Contr. Mort.(X)=		
Species		Slope= # Animals/Level= Temperature =		
Lab	____	48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	
Acc.		Comments:		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mor(X)= Sol. Con. Mor.(X)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	
Acc.		Comments:		
120-Hour EC_{50}		EC50 = 0.14 ppm (0.13-0.16) 95% C.L. + Moving Average Method Con. Mort.(X) = N/A Sol. Con. Mort.(X) = N/A	K.K. 01/25/91	Su
Species Skeletonema costatum	97.2%	Slope= N/A # Cells/mL = 10,000 (initial inoculum) Temp.= 20-22°C		
Lab Springborn Laboratories, Inc.		120-Hour Dose Level ppm/(% Inhibition) 0.033 (0), 0.065 (24), 0.13 (49), 0.25 (53), 0.50 (100)		
MRID # 416060-02		Comments: Based on nominal concentrations (active ingredient). % responses relative to solvent control.		